Centre for Biological Engineering

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/15	Version Number
		3.1

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.

Name(s) of reviewer: Qasim Rafiq	Date: 28/03/12
Signature: Q.R	

Amendments:

This is a risk assessment review of the biological risk assessment CBE/BRA/15. The title of the project is 'Development scalable and standardised manufacturing methods for human mesenchymal stem cells (hMSCs)'.

This project is hazard group 1 and there is no change in the risk for Qasim Rafiq. However this review has been conducted as there is the arrival of an 8-week miniproject Doctoral Training Centre student Thomas Heathman who will be working from 26/03/12 until 25/05/12. The risk is mitigated by the training and lab induction provided by Carolyn Kavanagh and Kulvindar Sikand (Lab Managers) covering biological spill response, waste disposal and general lab rules and procedures as well as relevant cell culture training from Qasim Rafiq. The risk is also mitigated by the fact that Thomas Heathman will be working **under supervision at all times** by Qasim Rafiq.

Qasim Rafiq has 3.5 years relevant cell culture and lab experience. 2.5 of those years have been working in the CBE level 2 facility.

Thomas Heathman has approximately 6 months experience of cell culture having completed an 8-week miniproject at Nottingham University as well as receiving cell culture training in the Bio Lab in the Chemical Engineering building at Loughborough University under the guidance of Kathryn Brosnan (Cell Culture Technician). Thomas's previous experience includes the following techniques:

- Extraction of primary mouse calvariae.
- Cryo-preservation of primary murine bone cells.
- reanimation of primary murine bone cells from cryo-preservation.
- Culture of primary murine bone cells (including passage, plating and media changing)
- Culture of murine embryonic stem cells (including passage and media changing)
- PLGA-PEG scaffold formation (inc. PLGA/PEG thermal blending and cyro-milling)
- Analysis using spectrophotometry
- Cell viability assays (MTS)
- Nitrite Assays
- ELISAs for Prostaglandin E2 synthasis.
- Immunocytochemistry for detecting osteopontin, osteocalcin, cadherin-11 and collagen-1
- Cell imaging (with and without staining) and subsequent image enhancement.
- Making-up media - Aseptic (osteogenic, with cytokines and with drugs diclofenac sodium and dexamethasone, antibotic free)

Description of the Experimental Procedures that Thomas Heathman will be involved in:

REVIEW DATE : 28/03/12

NEXT REVIEW DATE : 28/03/13

Preparation of culture medium:

500 ml of DMEM (Lonza) supplemented with 5.5 ml Ultra-Glutamin and 55 mL of FBS
Refer to additional SOPs 009

Routine cell culture:

The activity will involve basic cell culture activities including washing cells with trypsin, incubation for 4 minute after which cells will be spun down using a centrifuge and resuspended in culture media before being seeded into new flasks.

In addition to this other flasks within the incubator will have a media change and will involve aspirating spent media and topping up with fresh media before placing flasks in the incubator.

Daily samples will also be taken of any experimental flasks for analysis on the Nova Bioprofile FLEX analyser located in H23. A 1 ml sample will be taken from the flask and run on the bioanalyser.

Cell analysis:

Samples will be counted using the Nucleocounter (automated mammalian cell counter) and **not** Trypan blue.

Other cell analysis techniques will include preparing and running cell samples for flow cytometric analysis using the Quanta SC flow cytometer.

Spinner Flask culture:

Work will also include the preparation and culture of hMSCs on microcarriers using spinner flasks. The preparation of spinner flask culture requires use of the autoclave, however Thomas Heathman **will not** be using the autoclave at any time, and instead this will be done by Qasim Rafiq.

Description of the Experimental Procedures that Thomas Heathman will NOT be involved in:

Below is a list of procedures Thomas Heathman will not undertake as he is not trained to do so:

1. Use of the autoclave
2. Any handling or use of the liquid nitrogen stores
3. Any handling of Trypan Blue

This review or revision must be approved by the person supervising the work and the CBE Quality Manager. Significant changes may require a revised version of the risk assessment to be issued for re-approval by the local BGMSA and/ or the BSO and/or GM Safety Committee, as appropriate.	
Name of Approver: P. Hourd Position: CBE QM Signature: P Hourd	Date: 29/03/12.
Name of Approver: Position: Signature:	Date:
Name of Approver: Position: Signature:	Date:
Name of Approver: Position: Signature:	Date:

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/~~15~~ 15	Version Number
		2

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review/revision has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.

Name(s) of reviewer: Qasim Rafiq	Date: 05/01/12
Signature: Q.Rf	

Amendments:

This is an annual review of the biological risk assessment CBE/BRA/15. The title of the project is 'Development scalable and standardised manufacturing methods for human mesenchymal stem cells (hMSCs)'.

This project is hazard group 1 and there is no change in the risk for Qasim Rafiq. However this review coincides with the arrival of an 8-week miniproject Doctoral Training Centre student Arif Abed who will be working from 23/01/12 until 16/03/12. The risk is mitigated by the training and lab induction provided by Carolyn Kavanagh and Kulvindar Sikand (Lab Managers) covering biological spill response, waste disposal and general lab rules and procedures as well as relevant cell culture training from Qasim Rafiq. The risk is also mitigated by the fact that Arif Abed will be working **under supervision at all times** by Qasim Rafiq.

Qasim Rafiq has 3.5 years relevant cell culture and lab experience. 2.5 of those years have been working in the CBE level 2 facility.

Arif has ~ 1 year experience in cell culture having completed an MEng in Biochemical Engineering at University College London (UCL) which involved basic cell culture training and techniques.

Description of the Experimental Procedures that Arif Abed will be involved in:

Preparation of culture medium:

500 ml of DMEM (Lonza) supplemented with 5.5 ml Ultra-Glutamin and 55 mL of FBS
Refer to additional SOPs 009

Routine cell culture:

The activity will involve basic cell culture activities including washing cells with trypsin, incubation for 4 minute after which cells will be spun down using a centrifuge and resuspended in culture media before being seeded into new flasks.

In addition to this other flasks within the incubator will have a media change and will involve aspirating spent media and topping up with fresh media before placing flasks in the incubator.

Daily samples will also be taken of any experimental flasks for analysis on the Nova Bioprofile FLEX analyser located in H23. A 1 ml sample will be taken from the flask and run on the bioanalyser.

Cell analysis:

Samples will be counted using the Nucleocounter (automated mammalian cell counter) and **not**

REVIEW DATE: 05/01/12
NEXT REVIEW DATE: 05/01/13

Trypan blue.

Other cell analysis techniques will include preparing and running cell samples for flow cytometric analysis using the Quanta SC flow cytometer.

Spinner Flask culture:

Work will also include the preparation and culture of hMSCs on microcarriers using spinner flasks. The preparation of spinner flask culture requires use of the autoclave, however Arif Abed **will not** be using the autoclave at any time, and instead this will be done by Qasim Rafiq.

Description of the Experimental Procedures that Arif Abed will NOT be involved in:

Below is a list of procedures Arif Abed will not undertake as he is not trained to do so:

1. Use of the autoclave
2. Any handling or use of the liquid nitrogen stores
3. Any handling of Trypan Blue

This review or revision must be approved by the person supervising the work and the CBE Quality Manager. Significant changes may require a revised version of the risk assessment to be issued for re-approval by the local BGMSA and/ or the BSO and/or GM Safety Committee, as appropriate.	
Name of Approver: P Hurd	Date: 06/01/12
Position: CBE QM	
Signature: P Hurd	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	
Name of Approver:	Date:
Position:	
Signature:	

RISK ASSESSMENT REVIEW/REVISION RECORD

Risk Assessment Ref No:	CBE/BRA/15	Version Number
		1.0

This risk assessment should be reviewed **annually** or more frequently if there is any change in the work, or if new information becomes available that indicates the assessment may no longer be valid. **This form should be attached to the front of the current version of the risk assessment or to the new version of the risk assessment if one is issued**

The following review has been carried out on the dates indicated and either the assessment remains valid or it has been amended as indicated.	
Name(s) of reviewer: Qasim Rafiq	Date: 25/06/12
Signature: Q.Rf	

Reason for Review:

Involvement of additional personnel in Project (Section 4)

This project is hazard group 1 and there is no change in the risk for Qasim Rafiq. However this review has been conducted as there is the arrival of an 8-week miniproject Doctoral Training Centre student Jack Bridge who will be working from 18/06/12 until 10/08/12. The risk is mitigated by the training and lab induction provided by Carolyn Kavanagh and Kulvindar Sikand (Lab Managers) covering biological spill response, waste disposal and general lab rules and procedures as well as relevant cell culture training from Qasim Rafiq. The risk is also mitigated by the fact that Jack Bridge will be working **under supervision at all times** by Qasim Rafiq.

Qasim Rafiq has 3.5 years relevant cell culture and lab experience. 2.5 of those years have been working in the CBE level 2 facility.

Jack Bridge has approximately 6 months experience of cell culture having completed an 8-week miniproject at Nottingham University, an 8-week miniproject at Keele University as well as receiving cell culture training in the Bio Lab in the Chemical Engineering building at Loughborough University under the guidance of Kathryn Brosnan (Cell Culture Technician). Jack's previous experience includes the following techniques:

Over the last 6 months and 2 mini projects Jack has gained experience in cell culture and practiced the following things:

- Basic cell culture; feeding, passaging, cell counting, preparing media and PBS, freezing down/defrosting cells.
- Using different cell lines: C2C12, MRC5, primary foetal chick femur
- Seeding cells onto electrospun PET scaffolds
- Seeding cells into collagen gels
- Cell assays and staining including: trypan blue, Live/dead staining, MTT, Alizarin red, Bradford assay, Alamar blue, immunohistochemistry.

Other activities:

- Using the hydrostatic pressure bioreactor
- Measuring the oxygen tension of media
- Electrospinning random and aligned PET scaffolds
- Using the culture force monitor bioreactor

Description of the Experimental Procedures that Jack Bridge will be involved in:

Preparation of culture medium:

500 ml of DMEM (Lonza) supplemented with 5.5 ml Ultra-Glutamine and 55 mL of FBS
Refer to additional SOPs 009

Routine cell culture:

The activity will involve basic cell culture activities including washing cells with trypsin, incubation for 4 minute after which cells will be spun down using a centrifuge and resuspended in culture media before being seeded into new flasks.

In addition to this other flasks within the incubator will have a media change and will involve aspirating spent media and topping up with fresh media before placing flasks in the incubator.

Daily samples will also be taken of any experimental flasks for analysis on the Nova Bioprofile FLEX analyser located in H23. A 1 ml sample will be taken from the flask and run on the bioanalyser.

Cell analysis:

Samples will be counted using the Nucleocounter (automated mammalian cell counter) and **not** Trypan blue.

Other cell analysis techniques will include preparing and running cell samples for flow cytometric analysis using the Quanta SC flow cytometer.

Spinner Flask culture:

Work will also include the preparation and culture of hMSCs on microcarriers using spinner flasks. The preparation of spinner flask culture requires use of the autoclave, however Jack Bridge **will not** be using the autoclave at any time, and instead this will be done by Qasim Rafiq.

Description of the Experimental Procedures that Jack Bridge will NOT be involved in:

Below is a list of procedures Jack Bridge will not undertake as he is not trained to do so:

1. Use of the autoclave
2. Any handling or use of the liquid nitrogen stores
3. Any handling of Trypan Blue

Revision Required (Y/N)	**N**

If Yes, give details of the revision:

Approval:

Instructions for Reviewer:

1. *The completed form should be forwarded to the CBE Quality Manager. NOTE: Significant revision (See Guidelines GN006 & GN007) will require approval by the person supervising the work and subsequent review and approval by the original approving authority. This may require a revised version of the risk assessment to be issued for re-approval.*
2. *Where an annual review concludes that the risk assessment is still valid ie no revision is required, this should be recorded and the completed form forwarded to the CBE Quality Manager.*

Name of Approver: P. Hourd Position: CBE QM Signature:	Date: 25 June 2012
Name of Approver: Position: Signature:	Date:
Name of Approver: Position: Signature:	Date:
Name of Approver: Position: Signature:	Date:

CBE / BRA / 16 15
A. Chandra

Loughborough University

5 Jan 2012

For Health and Safety Unit Use only
ASSESSMENT NO.
CBE/BRA/15

RISK ASSESSMENT OF WORK WITH BIOLOGICAL MATERIALS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological materials must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials that may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTION(S) OF PART B, AND ALL OF PART C. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH AND SAFETY UNIT FOR REVIEW (VIA YOUR DEPARTMENTAL SAFETY OFFICER)
3. It is the responsibility of the Principal Investigator to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	29/01/2010	Date Approved:	02/02/2010

PART A: Please provide the following general information:

School/Department			
Chemical Engineering/ Centre for Biological Engineering (CBE)			
The Project			
Title of Project: 'Development scalable and standardised manufacturing methods for human mesenchymal stem cells'			
Project Reference Number: N/A			
Person responsible for this work (*Principle Investigator*):			
Name: Prof Chris J. Hewitt		Position: Professor of Biological Engineering	
Department: Centre for Biological Engineering		University School: Chemical Engineering	
Person conducting this assessment			
Name: Qasim Rafiq		Position: Research Student	
Department:	Chemical Engineering	Date Risk Assessment Undertaken:	20/01/2010
Proposed Project Start Date:		Proposed Project End Date:	01/10/2012

Assessment Review:
required at least once a year or immediately following any significant change to the project

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date					
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project *Brief yet clear outline only*

The potential of mesenchymal stem cells (MSCs) for therapeutic applications has generated widespread interest, however there are key issues which need to be addressed before the potential of MSCs as cellular therapies can be realised fully. Amongst them is the need to reproducibly obtain large numbers of well-characterised cells, which can only be achieved by defining the key parameters and critical to quality issues of scaled cell culture. Understanding these parameters will facilitate the development of a process to successfully scale-up the production of MSCs. This work seeks to address this challenge by developing a characterised, controlled and reproducible manufacturing-scale culture process for the standardised culture of adherent cell types including MSCs. The work will look to initially identify the parameters critical to quality for scaled cell cultivation and investigate novel surfaces for growing cells in stirred tank reactors. Work will include:

- Initially establishing a standardised set of process conditions for the expansion of human mesenchymal stem cells obtained from bone marrow in small scale culture including T-flasks and on microcarriers in spinner flasks.
- Translate these findings and demonstrate comparability to a larger scale bioreactor system (5L).

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations

1. **Preparation of culture medium:**
   500 ml of DMEM (Lonza) supplemented with 5.5 ml Ultra-Glutamin, 55 mL of FBS and 550 µl of gentamicin.
   Refer to additional SOPs 009
2. **Receiving cells & storing cells:**
   Cells will be shipped by Lonza Cologne AG in a liquid nitrogen transport box. Cells will be stored in the liquid nitrogen cryostorage unit.
   Refer to SOPs 005, 008, 013, 031, 032
3. **Thawing and initial growth:**
   Thaw frozen vial in a 37^{O}C water bath
   Transfer contents of the vial to T75 flask
   Initiate expansion by incubating at 37^{O}C, 5% CO_2.
   Allow culture to expand until 80% confluence is achieved – perform viable/non-viable cell counts.
   Subculture into further T75s using trypsin EDTA
   Refer to additional SOPs 006, 017, 024, 025
4. **Cell counting:**
   Stain a 100 µL sample of cell suspension with 100 µL of Trypan blue, mix and transfer 10µL to a haemocytometer. Count 3-4 large squares, take the average and multiply by the dilution factor, and then by 10,000 to give the number of cells/mL.
   The Quanta SC Flow cytometer will also be used to give more accurate cell counts once bioreactor culture commences.
   Refer to additional SOPs 029, 033, 034, 046
5. **Expansion using the 5L BIOSTAT Bplus bioreactor:**
   Following a run, the bioreactor is autoclaved for decontamination, cleaned manually and then autoclaved again for sterilisation.
   MSCs and 3-5L of media are added, and the parameters (stirrer speed, temperature, pH, pO_2 etc) set using the touchscreen system.
   Media is added/ removed using external pumps at set points during a fermentation cycle.
   Samples can be taken using the sample port at set times by using the syringe to extract media into the sample tube, and then transferring the sample aseptically to a sample bag or other container external to the bioreactor system.
   Sodium bicarbonate will be used to control the pH inside the bioreactor.
   Refer to additional SOP 078
   QR attended the training day organised by Sartorius engineers on how to use and maintain bioreactor on 08/10/09 as documented in QR's training file.

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: *micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).* ***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]***
Section 2: *cell cultures, tissues, blood, body fluids or excreta*
Section 3: *plants and plant material*
Section 4: *animals and animal tissues*

PART B: CELL CULTURES, TISSUES, BLOOD, BODY FLUIDS OR EXCRETA

B2.1 HAZARD AND RISK IDENTIFICATION: NATURE OF CELLS, TISSUES OR BODY FLUIDS

*This information gives an indication of the **potential** harm that the biological material may cause*

B2.1.1 List all cells or tissues to be used. *For cells indicate if primary, continuous or finite.*

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
Mesenchymal Stem Cells (finite)	Bone Marrow	Human	Lonza Cologne AG

B2.1.2 List all blood, body fluids or excreta to be used

Indicate in the adjacent box if Not Relevant (N/R)			N/R
Material type and ID	Organ Source	Species	From where will it be obtained?

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B2.1.4 Will material be screened for infectious agents (if from a cell culture collection answer B2.1.6)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, provide details of the types of screening and agents screened for: Refer to section B2.1.6	

B2.1.5 Will any clinical history (if relevant) be provided with this material?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes give details:	
If yes, will a policy of rejection of samples from diseased patients be adopted? Explain	
If yes, how will the information be disseminated in the course of the project?	
If yes, will this information be anonomised?	

B2.1.6 If obtained from a cell culture collection, is safety information provided?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If Yes, summarise here: The information supplied from Lonza states: - Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus and Hepatitis C Virus. - All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
Human Mesenchymal Stem Cells	Low	Well authenticated/characterised cell lines from commercial source. Cells have documented provenance of screening as described in section B2.1.6. Cells are categorised as hazard group 1 and as directed by supplied are to be handled in a containment level CL2 as a precautionary measure.
		If low risk or none proceed to section B2.2.4

see The Managing the risks in laboratories and healthcare premises – available at
http://www.hse.gov.uk/biosafety/biologagents.pdf

B2.2.2 If medium or high risk (section B2.2.1), name and classify the Biological Agents this material could be infected with. List the biological agents and indicate the ACDP hazard group classification*

Name of Agent	Classification

****see The Approved List of Biological Agents – available on the Health & Safety website or*** http://www.hse.gov.uk/pubns/misc208.pdf.

B2.2.3 Describe the routes of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
Details:				

B2.2.4 Are there any other biological hazards (other than adventitious infectious risk) associated with the materials e.g. tumourogenic cells

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If Yes, describe:	

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, Occupational Health must be consulted:	

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
If yes, identify the cells and the conditions these will grow: Initial culture will take place in T-flasks and will be fed DMEM complete medium and incubated at 37°C, 5%CO_2. As the investigation develops, work will be carried out using spinner flasks and may also include scale-up work using a 5L bioreactor (SOP078).	

B2.4.2 If culturing, will CD4+ cells be present. Describe what cells and for how long these cultures will be allowed to grow

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.4.3 If culturing, what is the maximum volume of culture grown?

Indicate in the adjacent box if Not Relevant (N/R)	
Per Flask	Per experiment
175 ml (T175 flask)	350 ml
100 ml (Spinner flask)	1L
5L (Bioreactor)	10L
15ml (Microbioreactor)	360mL

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES : Persons MUST NOT work with their own cells.

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	
If yes, has Ethics Committee approval been obtained:	

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.6.2 Will there be any other environmental risks?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.7 OTHER HAZARDS

B2.7.1 Are there any other hazards associated with this work? For example, hazardous chemicals, cryogenic gases ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes

If yes, identify these:

Dimethyl Sulphoxide (DMSO)
Trypan blue
Trypsin/EDTA
Liquid Nitrogen
Virkon
Industrial Methylated Spirit (IMS) – (COSHH form: "Ethanol, denatured")
OXYLITE – substance for protecting the pO_2 probe of the bioreactor
Antifoam – "Sigma Antifoam C"

If yes, have these been risk assessed and any necessary approval obtained?

Yes. All hazardous chemicals will be separately and individually evaluated under COSHH assessment.

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

Not required; the cell line is classified as Hazard group 1.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, provide details: The majority of the work will be carried out in Rooms H.25 (Mammalian cell lab) and H.27 (Microbial lab) within the CBE. Access to the Containment level 2 CBE lab unit is restricted to authorised workers with appropriate training in accordance with documented local Code of Practice and Quality Management System requirements for containment level 2 activities involving biological material. Outside of normal working hours the laboratories are locked to ensure safe storage of biological agents and unauthorised entry. Keys are only issued to authorised users who have been granted out of hours access following risk assessment of their intended work. There is no access to the laboratories by any cleaning or maintenance staff at any time unless a specific permit to work has been granted.	
(ii) Is access to the laboratory(s) to be used for this work restricted?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, provide details: Access is restricted to people with documented training (authorised access documented in each individual's training record) in accordance with the COP and QMS.	

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, list the sharps: Glass bioreactor vessel Cover slips for use with haemocytometers and microscopes	
If yes, justify their use – is there an alternative?: It is local practice in the CBE laboratory unit that the use of sharps is avoided wherever possible, with glass items replaced with plastic alternatives. However, the above sharps are essential for microscopy work (according to SOP033; "Use and Maintenance of Haemocytometer" and SOP080; "Use and Maintenance of Nikon TS100 Inverted Phase-Contrast Microscope". No suitable alternatives. The bioreactor vessel is made of glass and cannot be substituted. Plastic bottles (rather than glass) will be used to hold the corrective agents (antifoam or bicarbonate in this case; as described in SOP078 "Use and Maintenance of the 5L Biostat BPlus Bioreactor")	
If yes, describe there use and disposal: Used sharps are placed directly into a sharps containers conforming to BS 7320. Sharps bins are removed when three quarters full and contents rendered safe by autoclaving prior to their removal from site. Broken glass is placed in the sharps bins present in the laboratory. This will be done in accordance to SOP003 "Disposal of biological waste"	

If yes, describe any additional precautions employed to reduce risk:

Accident procedures for sharps and glass injuries are displayed in posters in all labs within the Unit. Safety glasses will be worn.

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker ie do the work procedures generate aerosols or splashes that pose a risk to workers?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, specify the type(s) and when they will be used:

A Class II Biological Safety Cabinet will be used for all culture work and manipulations that may produce aerosols or splashes but is primarily used to ensure protection of research materials as part of a quality assurance discipline. Procedures to be carried according to the following SOPs:

1) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC"

.

Appropriate personal protective equipment (PPE) including safety glasses and gloves are worn during bioreactor culture.

(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

If yes, specify:

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored?

T-flasks will be kept in 37°C incubators with 5%CO_2.

Spinner flasks will be kept in 37°C incubators with 5%CO_2 on magnetic platforms during initial cell expansion (SOP084). Cells will later be grown in closed, water-jacketed, sterile vessels and kept on the bench-top during larger-scale expansions (SOP078)

Material listed in B2.1.1 will be stored in a cryobank or temporary storage in designated cell culture incubators according to the following SOPs:

1) SOP005, "Storage and Transport of Biological Materials"
2) SOP008, "Receipt of Hazardous Biological Material"
3) SOP013, "Use and Maintenance of Liquid Nitrogen Stores"
4) SOP079, "Use and Maintenance of the Heracell Incubator"
5) SOP031, "Cryopreservation and Storage of Mammalian Cell Lines"

Storage units are located in Laboratories H25 of the CBE Laboratory Unit

How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills.

Cells will always be transferred in closed secondary containers large enough to carry the designated material. . Appropriate spill response procedures are posted in the lab and documented in detail in the following SOPs:

1) SOP005, "Storage and Transport of Biological Material"
2) SOP038, "Biological Spill Response"

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills

Transfer outside the CBE Laboratory Unit is not anticipated but any requirement is likely to be constrained within the University site. All transport will be subject to controlled procedures according to the local COP and SOP005 (see below). For example, if necessary, transfers will use double containment procedures Transport of research material between laboratories is done using sealed containers which are put into tube racks and trays and transported using trolleys according to the following SOPs. Waste potentially containing viable agents is not removed from the laboratories until it has been autoclaved.

1) SOP003, "Disposal of Biological Waste"
2) SOP005, "Storage and Transport of Biological Material"
3) SOP038, "Biological Spill Response"

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):	

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly?

Receiving of the hMSCs from Lonza Cologne AG will be carried out to ensure compliance to SOP008 "Receipt of Hazardous Biological Material".

Lonza will arrange the logistics of shipping and will be shipping the vials in a specially designed liquid nitrogen shipping container.

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

(ii) Where will these rotors/buckets be opened?

Sealed buckets will be opened on the bench within the Containment Level 2 (CL2) Laboratory Unit, unless there is evidence of a potential spillage, in which case the sealed buckets will be opened in the BSC (SOP009, "Use and Maintenance of HERASAFE KS Class II BSC",.

The centrifuge is operated and maintained according to the following SOPs:

1) SOP088, "Use and Maintenance of Eppendorf 5804 Centrifuge"
2) SOP038, "Biological Spill Response"

(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge

Procedures to prevent, contain and respond to leakages and spillages in the centrifuge are detailed in the following SOPs:

1) SOP088, "Use and Maintenance of Eppendorf 5804 Centrifuge"
2) SOP038, "Biological Spill Response"

Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory where a centrifuge is located to advise on spill response and reporting procedures.

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages.

Static incubators are used for initial culture, set at 37°C with 5% CO_2 (SOP079). A magnetic stirring system will be required for this stage of the cell culture (SOP084).

Procedures to prevent, contain and respond to spillages in the incubators are detailed in the following SOPs:

1) SOP079, "Use and Maintenace of Heracell CO2 Incubator"
2) SOP084, "Use and Maintenance of Spinner Flasks and Magnetic Stirrers"
3) SOP038, "Biological Spill Response"

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used:

The disinfectants were carefully chosen for effectiveness in use. The number of disinfectants in use is strictly limited to avoid errors and ambiguities in use and accidental mixing of compounds that may give rise to hazardous reactions or the formation of toxic products. Unless there are compelling reasons to do otherwise, Virkon (1% w/v) is the sole disinfectant used in the laboratories other than 70% IMS which is used for general disinfection cleaning (SOP004) where Virkon cannot be used; for example stainless steel surfaces.

Virkon has a wide range of bactericidal, virucidal, fungicidal and sporocidal activities. Representative viruses from all the major virus families are inactivated by Virkon. Working solutions of 1% w/v have low toxicity and no irritancy. Selection and procedures detailed in the following SOPs:

1) SOP004, "General Laboratory Housekeeping"
2) SOP006, "Selection and Use of Virkon Disinfectant"

3) SOP039, "Storage, Handling and Disposal of Chemicals"

COSHH Risk Assessment reference for Virkon SAF/MM/1745. The Bioreactor will also be cleaned using Virkon according to SOP078.

Have these disinfectants been validated for use with the recipient biological material?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes

If yes, describe the procedure:

They are well known to be effective disinfectants against a wide range of viruses, fungi and bacteria. For Hazard Group 1 it is sufficient to rely on the manufacturers data, providing the recommended concentrations and contact times are used. Hence Virkon (1%) is used as per manufacturers instruction and according to standard procedures detailed in the COP and the following SOP:

1) SOP006, "Selection and Use of Virkon Disinfectant"

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored?

Side fastening Howie type lab coats are worn. They are stored outside the laboratories in purposely designed change rooms. Proper use of PPE is described in the following SOP: SOP037, "Use of Personal Protective Equipment (PPE)".

(ii) What type of gloves will be worn and where will they be stored?

1. Autoclave gloves, which will be stored in close proximity to the autoclave equipment in the Autoclave Room (H31).
2. Cryogenic gloves, which will stored in close proximity to the Liquid Nitrogen storage containers located in Gas Pod 3, Analytical Lab (H23)
3. Latex powder free gloves for general use, which will be stored in the change rooms and point of entry to each laboratory within the CBE Laboratory Unit.

Correct use of PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

(iii) Describe any other PPE to be used:

1. Laboratory safety glasses (including those for spectacle wearers)
2. Face Shields (primarily for handling liquid nitrogen)
3. Shoe covers, in case of a spillage
4. Aprons or disposable lab coats for extra protection over Howie type laboratory coat.

Correct use of the above PPE is described in SOP037, "Use of Personal Protective Equipment (PPE)"

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

1. A Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
2. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

If yes, describe:

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?		
	Treatment before disposal	Validation
Liquid waste	If removed from bioreactor: Virkon sterilise (SOP003 – Disposal of biological waste) If contained in bioreactor: Autoclave sterilise. Autoclave number: CBE-045 must be used as it has a mechanical aid for loading the bioreactor vessel (SOP025).	According to manufacturers instructions; see section C1.2.9 Treatment Cycle (6) "Sterilisation and Disposal of Liquid Waste" - validated according to SOP025, " Use and maintenance of the Systec Autoclave"
Solid waste	Autoclave sterilise (SOP003 – disposal and disinfection of biological waste) Autoclave number: CBE-045 must be used as it has a mechanical aid for loading the bioreactor vessel (SOP025).	Treatment Cycle (1) "Solids, instruments" - validated according to SOP025, " Use and maintenance of the Systec Autoclave"

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box			
	Type of waste	*Autoclave cycle (temp, cycle time)*	*Treatment monitor*
Liquid waste	Culture media, containing cells following reactor run	121°C, 15 minute cycles. Treatment cycle (6) "Sterilisation and Disposal of Liquid Waste"	A bottle of water containing a probe is run along with the waste
Solid waste	Cell culture consumable	121°C for 15 minutes. Treatment cycle (2) "Solid Laboratory Waste"	Designated Autoclave tape monitors

Location of autoclave	*Servicing details*	*Location of back-up autoclave*	*Designated area for storage of unsterilised waste*
Autoclave CBE-045 in Autoclave Room (H31) within the CBE Laboratory Unit i.e. same location as intended work	Annual	Autoclave CBE-044 in Autoclave Room (H31) or Systec Autoclave in Automated Cell Culture Suite (H22).	In secure cage within the Autoclave Room (H31)

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain? Media will disposed of by the drain with copious amounts of water - smaller volumes will be sterilised using Virkon in accordance with SOP003 – "Disposal of biological waste". Larger volumes autoclaved before being discarded.
As solid waste?
Other?

C1.2.16 Solid Waste Disposal
Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

European Waste Catalogue Code	Categorisation		Disposal Method
		Hatch relevant box(es)	
18 01 01	Sharps	X	Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
18 01 02 [human]	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins>incineration only
18 01 02 [animal]	Animal body carcases or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.16 of this RA in which case they must be pre-treated before disposal and classified 18 01 04 [sealed bins])		Rigid one way sealed tissue bins > incineration only
18 01 03	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
18 01 04 [bags]	Infected or potentially infected lab wastes that have been pre treated before leaving the site	X	Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow clinical waste bags > clinical waste disposal (incineration)
18 01 04 [sealed bins]	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site		Disinfection or sterilisation (as identified in C1.2.13) in the lab suite > placement in yellow one way sealed tissue bins > incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	
(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	N/R

Provide details of the training required:

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a fermenter be used to culture cells?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, describe the size, and type of the fermenter. 2 x 5L BIOSTAT B plus stirred-tank fermenters used to culture hMSCs cells.	
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe:	

C1.2.19 Other Control Measures Required?

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC: Procedures for dealing with small and large spillages are detailed in the following SOPs: 1) SOP006, "Selection and use of Virkon Disinfectant" 2) SOP009, "Use and Maintenance of HERASAFE KS Class II BSC" 3) SOP038, "Biological Spill Response" 4) SOP052, "Use and Maintenance of Bioquell Advanced Microflow Biosafety Cabinet" Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit where a BSC is located to advise on spill response (inside the BSC) and reporting procedures.
Within the laboratory but outside the control measure e.g. BSC, spill tray Procedures for dealing with small and large spillages are detailed in the following SOPs: 1) SOP006, "Selection and use of Virkon Disinfectant" 2) SOP038, "Biological Spill Response" Labelled Biological Spill kits are located in each laboratory within the CBE Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest biological (and chemical) spill kits. Posters are also displayed in each laboratory within the Unit to advise on spill response (ouside the BSC) and reporting procedures.
Outside the laboratory e.g. during transport Procedures for dealing with small and large spillages are detailed in the COP and the following SOPs: 1) SOP005, "Storage and Transport of Biological Material" 2) SOP006, "Selection and use of Virkon Disinfectant" 3) SOP038, "Biological Spill Response

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

1. Procedures to respond to accidental exposure are detailed in SOP038, "Biological Spill Response" and the local COP. These are detailed in spill response posters located in each laboratory within the Unit. Accident procedures in the case of glass or sharps injury are described in the local COP and displayed in posters located in each laboratory within the Unit
2. Designated hand washing facilities are located in each laboratory change room and in the Analytical Laboratory (H23).
3. Eye Wash stations are located next to each 'hand washing only' sink in each laboratory change room and in the Analytical Laboratory (H23).
4. A First Aid Kit is located outside the Laboratory Unit. Signs are posted throughout the Laboratory Unit to enable workers to locate the nearest Medical Kit. Contact details for First Aiders are posted in each laboratory within the Unit
5. Essential and Emergency Contact details are posted in each laboratory within the Unit.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work?

The work activities within this project involve biological agents (BAs) assessed as Hazard Group 1. However, all procedures will be carried out under Containment level 2 (CL2) within the CL2 CBE Laboratory Unit. This project, involving the use of Hazard Group 1 BAs that require Containment Level 1 are carried out at Containment Level 2 for reasons other than worker protection; this includes the need to ensure research material protection (e.g. the use of a class II safety cabinet) and to impose a quality assurance discipline.

C2.2. Describe extra controls or derogation from certain controls:

None

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
CBE Laboratory Unit (*self contained suite of laboratories and ancillary rooms within the CBE*)	Centre for Biological Engineering	Loughborough Holywell Park	Carolyn Thomas Bob Temple Chris Hewitt

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	ID	Position
Rafiq	QR	A818033	Research student
Coopman	KC	5011598	Lecturer
Hewitt	CJH		Professor

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

Identified personnel are trained in required procedures and equipment. Formal records of training are kept for all workers authorised to work at Containment Level 2 (CL2) within the CBE CL2 Laboratory Unit. Instruction against local Code of Practice and QMS

KC and CJH are the main supervisors for the project but will not be participating in practical work. All practical work carried out by QR is subject to conclusions recorded in training file.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Hewitt	>20 years cell culture, microbiological and aseptic technique working to BSc and PhD level.
Coopman	PhD in Pharmacology including >5 years experience in cell culture and aseptic technique. Documented in Training Record
Rafiq	MEng in Biochemical Engineering with ~1 years experience in cell culture and aseptic technique. Documented in Training Record

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:

NONE: Cleaners and Maintenance workers are not authorised to enter the laboratory. All laboratory cleaning is undertaken by authorised personnel (ie CBE staff). Access for non-laboratory workers is subject to a local permit-to-work procedures. If access is needed for essential maintenance of equipment for example a clean down and decontamination of the laboratories will be performed. This will be documented with decontamination certificates and the maintenance worker fully supervised according to SOP004 " General Laboratory Houskeeping" and the local Code of Practice Two laboratory shut downs occur every year for a week for maintenance work to be done in the CBE Laboratory Unit. Prior to these shut down weeks a full deep clean decontamination will be performed in the all laboratory areas.

All other workers in the CBE Laboratory Unit are authorised personnel.

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

Yes – Hepatitis B vaccination

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

No

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
Approval number:	
Date obtained:	Ethics committee name:

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
NOTE: The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. See the DEFRA website for details. UNLESS THIS SECTION IS NOT RELEVANT (N/R) ie THE INTENDED WORK DOES NOT USE ANIMAL PRODUCTS - CONSULT THE LU H&S OFFICE TO REVIEW APPLICATION REQUIREMENTS BEFORE ANY SUBMISSIONS	

8. DECLARATION

*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and*

University Biological Safety Officer

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- accept that for some Containment Level 2 and all CL3 activities **a statutory notification period of 20 days** may be required before work can commence
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

Name: Person conducting assessment	**Signature**	**Date**
Qasim Rafiq	Q. Rafiq	29/01/10
Name: Other signature (s) (if required – please state position)		
Paul Hourd	P Hourd	29/01/10
Name: Principal Investigator	**Signature**	**Date**
Chris J. Hewitt	C J Hewitt	2/02/10.

9.APPROVAL		
Name: Departmental Safety Officer	**Signature**	**Date**
Chris J. Hewitt	C J Hewitt	2/02/10.
Name: University Biological Safety Officer	**Signature**	**Date**
NA		

Lonza Walkersville, Inc.
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scientific.support@lonza.com
Scientific Support: 800-521-0390
Document # AA-2501-14 06/09
Walkersville, MD 21793-0127 USA

Poietics® Human Mesenchymal Stem Cells

Instructions for Use

Safety Statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use *in vitro* diagnostic or clinical procedures.

WARNING: CLONETICS® AND POIETICS® PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing cannot offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Scientific Support.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. BulletKit® Instructions: Upon arrival, store basal medium at 4°C to 8°C and SingleQuots® at –20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4°C and added to basal medium within 72 hours of receipt. After SingleQuots® are added to basal medium, use within one month. Do not re-freeze.
   Using media or reagents other than what's recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.
4. Trypsin/EDTA Solution has a limited shelf life or activation at 4°C. If, upon arrival, Trypsin/EDTA is thawed, immediately aliquot and refreeze at –20°C.

NOTE: To keep Trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at –20°C.

Preparation of Media

1. Decontaminate the external surfaces of the MSCGM™ SingleQuots® Cryovials and the Mesenchymal Stem Cell Basal Medium (MSCBM) bottle with 70% v/v ethanol or isopropanol.
2. Aseptically open the bottle of Mesenchymal Cell Growth Supplement (MCGS). Add the contents to the 440 ml MSCBM.
3. Aseptically open each cryovial of L-Glutamine and GA-1000 and add the entire amount from each SingleQuots® to the MSCBM.
4. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses should not affect the cell characteristics.
5. Use supplemented medium for the maintenance of hMSC only.

Thawing of Cells / Initiation of Culture Process

1. The recommended seeding density for Human Mesenchymal Stem Cells is 5,000-6,000 cells per cm^2.
2. To set up cultures calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. Add the appropriate amount of medium to the vessels (0.2 - 0.4 ml per cm^2) and allow the vessels to equilibrate in a 37°C, 5% CO_2 humidified incubator for at least 30 minutes.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath.
4. Watch your cryovial closely; when the last sliver of ice melts remove it. Do not submerge it completely. Thawing the cells for longer then 1 ½ minutes results in less than optimal results.
5. Remove the cryovial immediately, wipe it dry, and transfer to a sterile field where the equilibrated flasks should be waiting, ready to

seed. Rinse the cryovial with 70% alcohol, and then wipe to remove excess.

6. Using a micropipet, gently add the thawed cell suspension to 5 ml of temperature-equilibrated medium.
7. Centrifuge at 500 x g for 5 minutes at room temperature.
8. Resuspend the pellet in a minimum volume of temperature equilibrated Mesenchymal Stem Cell Growth Medium (MSCGM™) by gently pipetting up and down. Count the total number of viable cells.
9. Add the calculated volume of cell suspension to each prepared flask and gently rock to disperse the cell suspension over the growth surface.
10. Incubate at 37°C, 5% CO_2 and 90% humidity.

Subculturing

1. Aseptically remove and discard all of the spent media from the flasks.
2. Wash the attached cell layer with Dulbecco's Phosphate Buffered Saline or an equivalent calcium and magnesium free balanced salt solution. Add the wash solution to the side of the flask opposite the attached cell layer. Rinse by rocking the flask back and forth several times. Aseptically remove and discard the wash solution.
3. Add a sufficient volume of Clonetics® Trypsin-EDTA (CC-3232) solution to cover the cell layer (approx. 0.05 ml/cm^2). Gently rock the flask(s) to ensure that the cells are covered by the trypsin solution. Incubate at room temperature for five minutes, then observe under a microscope. If the cells are less than 90% detached, continue incubating and observe every 3 minutes. Tapping the flask or plate will expedite cell detachment. Do not incubate the cells longer than 15 minutes.
4. Once ≥90% of the cells are rounded and detached, stand the flasks on end for a minimal length of time to allow the cells to drain. Add an equal volume of temperature equilibrated MSCGM™ to each vessel. Disperse the solution by pipetting over the cell layer surface several times.
5. To remove the trypsin, centrifuge cells at approximately 600 x g for five minutes at room temperature.
6. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated MSCGM™ and remove a sample for counting.
7. Count the cells with a hemacytometer or cell counter and calculate the total number of cells. Make a note of your cell yield for later use.
8. If necessary, dilute the suspension with MSCGM™ to achieve the desired "cells/ml" and re-count the cells.
9. Assess cell viability using Trypan Blue.
10. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}$$

11. Determine the total number of flasks to inoculate by using the following equation. The number of flasks needed depends upon cell yield and seeding density.

$$\text{Total \# of Flasks to innoculate} = \frac{\text{Total \# of viable cells}}{\text{Growth area} \times \text{Rec. Seeding Density}}$$

12. Use the following equation to calculate the volume of cell suspension to seed into your flasks. Determine the volume of MSCGM™ to add to each flask so that the final culture volume is 0.2 – 0.4 ml per cm^2.

$$\text{Seeding Volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 11}}$$

13. Prepare flasks by labeling each flask with the passage number, strain number, cell type and date.
14. Add the appropriate volume of temperature equilibrated MSCGM™ as determined in step 12.
15. Incubate at 37°C, 5% CO_2 and 90% humidity.
16. Three to four days after seeding, completely remove the medium. Replace with an equal volume of MSCGM™. Cultures will be near confluence by day 6 or 7 and ready to subculture.

Maintenance

1. hMSC cultures should be fed 3-4 days after plating.
2. To feed the cultures, gently and completely remove the MSCGM™ from the culture vessel.
3. Replace with an equal volume of temperature equilibrated MSCGM™ and return the culture vessels to the incubator.
4. When seeded at 5,000-6,000 cells per cm^2 of surface area, hMSC should be near confluence by day 6 or 7. The hMSC are contact-inhibited and should be subcultured when they are just sub-confluent (approximately 90% confluent). Cells are frozen in passage two and we recommend they are used by passage 5.

Adipogenic Assay Procedure

Prepare Adipogenic Induction Medium

Adipogenic Induction Medium should be used once the hMSC have become 100% confluent (approximately 5-13 days). Prepare the medium before hMSC become confluent.

1. Decontaminate the external surfaces of the hMSC Adipogenic Induction Medium (PT-3102B) and the following SingleQuots® with 70% v/v ethanol or isopropanol:
    a. h-Insulin (recombinant)
    b. L-glutamine
    c. MCGS
    d. Dexamethasone
    e. Indomethacin
    f. IBMX (3-isobuty-l-methyl-xanthine)
    g. GA-1000
2. Aseptically open the above SingleQuots® and add the contents to the 170 ml of Adipogenic Induction Medium.
3. Rinse each SingleQuots® Vial with the medium. It may not be possible to recover the entire contents of each SingleQuots®. Small losses should not affect the cell characteristics.
4. Use supplemented medium for the adipogenic induction of hMSC only. Store at 2°C to 8°C in the dark until needed.

Prepare Adipogenic Maintenance Medium

1. Decontaminate the external surfaces of the hMSC Adipogenic Maintenance Medium (PT-3102A) and the following SingleQuots® with 70% v/v ethanol or isopropanol:
    a. h-Insulin (recombinant)
    b. L-Glutamine
    c. MCGS
    d. GA-1000
2. Aseptically open the above SingleQuots® and add the contents to the 170 ml of Adipogenic Maintenance Medium.
3. Rinse each SingleQuots® Vial with the medium. It may not be possible to recover the entire contents of each SingleQuots®. Small losses should not affect the cell characteristics.
4. Store supplemented Adipogenic Maintenance Medium at 2°C to 8°C in the dark until needed.

Adipogenesis Culture Protocol

1. Plate 2.1 x 10^4 hMSC per cm^2 of tissue culture surface area in 0.2 to 0.3 ml of MSCGM™ per cm^2 of tissue culture surface area.

For example: 2 x 10^5 cells in 2 ml medium per 9.6 cm^2 well of a 6 well plate. Incubate the cells at 37°C, in a humidified atmosphere of 5% CO_2.

2. Feed the cells every 2-3 days by completely replacing the medium with fresh MSCGM™ until the cultures reach confluence (5-13 days). hMSC must be confluent, or post confluent, for optimal adipogenic differentiation.
3. At 100% confluence, three cycles of induction/ maintenance will stimulate optimal Adipogenic differentiation. Each cycle consists of feeding the hMSC with supplemented Adipogenesis Induction Medium and culture for 3 days (37°C, 5% CO_2) followed by 1-3 days of culture in supplemented Adipogenic Maintenance Medium. Feed non-induced control hMSC with only supplemented Adipogenic Maintenance Medium on the same schedule. Adipogenic cells are delicate and care should be used to avoid disrupting the numerous lipid vacuoles in the cells. Do not let the cells dry out when changing medium.
4. After 3 complete cycles of induction/ maintenance, culture the hMSC for 7 more days in supplemented Adipogenic Maintenance Medium, replacing the medium every 2-3 days.
5. The extent of adipogenic differentiation may be noted by microscopic observation of lipid vacuoles in the induced cells. To document the adipogenic differentiation, cultures may be assayed using AdipoRed™ Assay Reagent. Non-induced cells will have few, if any, lipid vacuoles.

Chondrogenic Assay Procedure

Prepare Incomplete Chondrogenic Induction Medium

1. Decontaminate the external surface of the Differentiation Basal Medium – Chondrogenic and the following SingleQuots® with 70% v/v ethanol or isopropanol.
    a. Dexamethasone
    b. Ascorbate
    c. ITS + Supplement
    d. GA-1000
    e. Sodium Pyruvate
    f. Proline
    g. L-Glutamine
2. Aseptically open the above SingleQuots® and add the contents to the 185 ml of Differentiation Basal Medium – Chondrogenic to prepare the Incomplete Chondrogenic Induction Medium.
3. Rinse each SingleQuots® Vial with the medium. It may not be possible to recover the entire

content of each SingleQuots®. Small losses should not affect cell characteristics.

4. Store the Incomplete Chondrogenic Induction Medium at 2°C to 8°C in the dark until needed.

Prepare and Aliquot TGF-β3

1. Resuspend the lyophilized TGF-β3 (Lonza PT-4124) with sterile 4mM HCl supplemented with 1 mg/ml BSA or HSA to a concentration of 20 µg/ml. For example, use 100 µl diluent for 2 µg of TGF-β3.

Note: Each µl of TGF-β3 will convert 2 ml of Incomplete Chondrogenic Medium into Complete Medium.

2. Aliquot small volumes of TGF-β3 into freezer-safe tubes and store at less than –70°C for no more than 6 months. (For example, the TGF-β3 can be frozen in 5 µl aliquots. Each aliquot will be sufficient to supplement 10 ml of Incomplete Chondrogenic Induction Medium.)

Complete Chondrogenic Induction Medium

1. After thawing, the aliquot of TGF-β3 may need to be centrifuged briefly at low speed to pull the small volume (e.g. 5 µl) to the bottom of the tube.
2. Pipette the volume of Incomplete Chondrogenic Induction Medium that you intend to supplement (e.g. 10 ml) into a tube.
3. To recover the full volume of TGF-β3, transfer 100 µl of this Incomplete Chondrogenic Medium to the tube of TGF-β3.
4. Mix the solution by pipetting and transfer it back to the tube of Chondrogenic Induction Medium. Repeat this process to be certain that you have recovered the TGF-β3. Cap and invert several times to mix.
5. The Chondrogenic Induction Medium is now Complete, and contains TGF-β3 at a final concentration of 10 ng/ml.

Note: *Complete Chondrogenic Medium must be prepared fresh and used within 12 hours.*

Chondrogenesis Culture Protocol:

1. Calculate the total number of pellet cultures required for your experiment (2.5 x 10^5 hMSCs are needed to form each chondrogenic pellet). Transfer this amount of cells to an appropriate culture tube to wash the cells.
2. Wash the hMSCs with **Incomplete** Chondrogenic Medium: Centrifuge the cells at 150 x g for 5 minutes at room temperature, and aspirate/ discard the supernatant. Resuspend the cells in 1 ml **Incomplete** Chondrogenic Medium per 7.5 x 10^5 cells, centrifuge again at 150 x g for 5 minutes and aspirate/discard the medium.
3. Resuspend the hMSCs in **Complete** Chondrogenic medium to a concentration of 5.0 x 10^5 cells per ml.
4. Aliquot 0.5 ml (2.5 x 10^5 cells) of the cell suspension into 15 ml polypropylene culture tubes. Centrifuge the cells at 150 x g for 5 minutes at room temperature. DO NOT aspirate the supernatant or resuspend the pellet.

   NOTE: Polypropylene tubes are used so that the cells do not adhere to the tube. Polystyrene tubes should not be used.
5. Loosen the caps of the tubes one half turn to allow gas exchange and incubate the tubes at 37°C, in a humidified atmosphere of 5% CO_2. Do not disturb the pellets for 24 hours.
6. Feed the cell pellets every 2-3 days by completely replacing the medium in each tube (to avoid aspirating the pellets when aspirating the medium, attach a sterile 1-200 µl pipette tip to the end of the aspirating pipette). Add 0.5 ml of freshly prepared Complete Chondrogenic Medium to each tube.
7. After replacing the medium, flick the bottom of each tube to ensure that the pellet is free-floating, loosen the caps and return the tubes to the 37°C incubator.
8. Chondrogenic pellets should be harvested after 14 to 28 days in culture. Pellets may be formalin fixed and paraffin embedded for histological processing or may be prepared for frozen sectioning. Thin sections may be slide-mounted and stained for glycosaminoglycans with Safranin O or may be immunostained for Type II collagen.

Osteogenic Assay Procedure

Prepare Osteogenic Induction Medium

1. Decontaminate the external surfaces of the hMSC Differentiation Basal Medium – Osteogenic and the following SingleQuots® with 70% v/v ethanol or isopropanol:
   a. Dexamethasone
   b. L-Glutamine
   c. Ascorbate
   d. Pen/Strep
   e. MCGS
   f. β-Glycerophosphate
2. Aseptically open the above SingleQuots® and add the contents to the 170 ml of hMSC Differentiation Basal Medium – Osteogenic.

3. Rinse each SingleQuots® Vial with the medium. It may not be possible to recover the entire contents of each SingleQuots®. Small losses should not affect the cell characteristics.
4. Store the supplemented Osteogenic Differentiation Medium at 2°C to 8°C in the dark until needed.

Osteogenesis Culture Protocol:

1. Plate 3.1 x 10^3 hMSCs per cm^2 of tissue culture surface area in 0.2-0.3 ml of MSCGM™ per cm^2 tissue culture area. For example: 3 x 10^4 cells in 2 ml medium per 9.6 cm^2 well of a 6-well plate.
2. Allow the cells to adhere to the culture surface for 4 to 24 hours in MSCGM™ at 37°C, in a humidified atmosphere of 5% CO_2.
3. Induce Osteogenesis by replacing the MSCGM™ with Osteogenesis Induction Medium.
4. Feed the induced hMSCs every 3-4 days for 2-3 weeks by completely replacing the medium with fresh Osteogenesis Induction Medium. Feed non-induced control hMSCs with MSCGM™ on the same schedule.
5. Osteogenic induced cells will show changes in cell morphology, from spindle shaped to cuboidal shaped, as they differentiate and mineralize. Gaps may form in the post confluent cell layer and cells may begin to delaminate from culture surface. If this de-lamination is observed, proceed immediately to analysis of osteogenic differentiation as indicated by calcium deposition, or use the induced cells for other assays requiring osteocytes.
6. For calcium deposition assays, harvest cells by rinsing them in calcium free PBS, then scraping cells from the culture surface in the presence of 0.5M HCl. Assay the extracts from osteogenic induced cultures for calcium content and compare to extracts from non-induced control cells.

Ordering Information

PT-2501	hMSC - Human Mesenchymal Stem Cells	≥750,000 cells

Related Products

Mesenchymal Stem Cell Growth Medium

PT-3001	MSCGM™ BulletKit®	MSCBM (500 ml) plus SingleQuots® of growth upplements
PT-3238	MSCBM	Mesenchymal Stem Cell Basal Medium (440 ml)

PT-4105	MSCGM™ SingleQuots®	Formulates MSCBM to MSCGM™. Contains Mesenchymal Cell Growth Supplement (MCGS), L-glutamine, and GA-1000.

Differentiation Media BulletKits – Adipogenic

PT-3004	hMSC Differentiation BulletKit ® – Adipogenic	Contains Adipogenic Maintenance Medium (170 ml), Adipogenic Maintenance SingleQuots® Kit, Adipogenic Induction Medium (170 ml), and Adipogenic Induction SingleQuots® Kit
PT-3102B	Adipogenic Induction Medium	170 ml
PT-3102A	Adipogenic Maintenance Medium	170 ml
PT-4122	hMSC Adipogenic Maintenance SingleQuots® Kit	Frozen supplements and growth factors. (h-Insulin (Recombinant), L-glutamine, Mesenchymal Cell Growth Supplement (MCGS), and GA-1000.)
PT-4135	hMSC Adipogenic Induction SingleQuots® Kit	Frozen supplements and growth factors. (h-Insulin (Recombinant), L-glutamine, Mesenchymal Cell Growth Supplement (MCGS), GA-1000, Dexamethasone, Indomethacin and IBMX.)
PT-7009	AdipoRed™ Assay Reagent	5 x 4.0 ml

Differentiation Media BulletKit® – Chondrogenic

PT-3003	hMSC Differentiation BulletKit® – Chondrogenic	Contains Differentiation Basal Medium – Chondrogenic (185 ml) and hMSC Chondrogenic SingleQuots® Kit.
PT-3925	Chondrogenic Basal Medium	185 ml
PT-4121	hMSC Chondrogenic SingleQuots® Kit	Supplements and growth factors (ITS + Supplement, Dexamethasone, Ascorbate, Sodium Pyruvate, Proline, GA-1000, L-glutamine)
PT-4124	TGF-β3	1 vial of lyophilized TGF-β3, 2 µg *(Required Chondrogenic Differentiation Reagent)*

*Note: TGF-β3 is supplied as a separate part from the hMSC Chondrogenic SingleQuots® Kit

Differentiation Media BulletKit® - Osteogenic

PT-3002	hMSC Differentiation BulletKit® – Osteogenic	Contains Differentiation Basal Medium – Osteogenic (170 ml), and hMSC Osteogenic SingleQuots®.
PT-3924	Osteogenic Basal Medium	170 ml
PT-4120	hMSC Osteogenic SingleQuots®	Supplements and growth factors (Dexamethasone, Ascorbate, Mesenchymal Cell Growth Supplement (MCGS), L-glutamine, Penicillin/Streptomycin, β-Glycerophosphate)
CC-3232	Trypsin/EDTA Solution	100 ml

Product Warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells only if Poietics® Media are used, and the recommended protocols are followed. Cryopreserved hMSC are assured to be viable and functional when thawed and maintained properly.

Quality Control

HIV-1, Hepatitis B and Hepatitis C are not detected for all donors and/or cell lots. All cells are performance assayed and test negative mycoplasma, bacteria, yeast and fungi. Cell viability and morphology are measured after recovery from cryopreservation. For detailed information concerning QC testing, please refer to the Certificate of Analysis.

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